Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine *in vitro*

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Abstract

Gomes BPFA, Sauza SFC, Ferraz CCR, Teixeira FB, Zaia AA, Valdrighi L, Souza-Filho FJ. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine *in vitro*. *International Endodontic Journal*, **36**, 267–275, 2003.

Aim To evaluate the effectiveness of 2% chlorhexidine gluconate gel and calcium hydroxide ($Ca(OH)_2$) as intracanal medicaments against *Enterococcus faecalis*.

Methodology One hundred and eighty dentine tubes prepared from intact freshly extracted bovine maxillary central incisors were infected *in vitro* for 7 days with *E. faecalis*. The specimens were divided into four groups, according to the intracanal medicament used, as follows: Group 1: 2% chlorhexidine gluconate gel; Group 2: calcium hydroxide in a viscous vehicle (polyethyleneglycol 400); Group 3: 2% chlorhexidine gluconate gel + calcium hydroxide and Group 4: Brain Heart Infusion (BHI) broth (control group). The medicaments were placed into the canal lumen and left there for experimental times of 1, 2, 7, 15 and 30 days. After each period, irrigation with sterile saline to remove the medicament was performed and the canals were dried with sterile paper points. Dentine chips were removed from the canals with sequential sterile round burs at low speed. The samples obtained with each bur were immediately collected in separate test tubes containing BHI broth. The tubes were incubated at 37°C and daily observed for microbial growth, visualized by the medium turbidity.

Results Chlorhexidine gel alone completely inhibited the growth of *E. faecalis* after 1, 2, 7 and 15 days. Calcium hydroxide allowed microbial growth at all experimental times. The combination of chlorhexidine and $Ca(OH)_2$ was effective after 1 and 2 days demonstrating 100% antibacterial action; however, its antibacterial activity reduced between 7 and 15 days.

Conclusions Under the conditions of this study, it can be concluded that 2% chlorhexidine gel alone was more effective against *E. faecalis* than calcium hydroxide ($P < 0.05$). However, its antibacterial activity depended on how long it remained inside the root canal.

Keywords: bacteria, bovine teeth, calcium hydroxide, chlorhexidine, intracanal medicaments.

Received 11 March 2002; accepted 16 October 2002

Introduction

One of the most important objectives of root canal treatment is the elimination of microorganisms from the root canal system.

Anaerobic bacteria, especially black-pigmented Gram-negative species, have been linked to the signs and symptoms of periapical disease (Gomes *et al.* 1994, 1996a). Facultative bacteria such as *Enterococcus faecalis*, have also been isolated from infected root canals and may be related to failure of root canal treatment (Engström 1964, Cavalleri *et al.* 1989, Gomes *et al.* 1996b, Molander *et al.* 1998, Peciuliene *et al.* 2000, Pinheiro *et al.* in press).

Although chemo-mechanical preparation of root canals is able to reduce the number of bacteria, an

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intracanal medicament with antibacterial action is required to maximize the disinfection of the root canal system in infected cases (Byström *et al.* 1985). The need for medication increases, especially in those cases where an infection is resistant to regular treatment, and the therapy cannot be successfully completed due to the presence of pain or continuing exudate (Spangberg 1994). For this reason, a wide variety of intracanal medicaments have been used, such as calcium hydroxide ($Ca(OH)_2$) pastes and chlorhexidine gels.

Calcium hydroxide plays an important role in endodontics through its ability to induce hard tissue formation, its moderate antibacterial action and its tissue-dissolving capability (Nerwich *et al.* 1993). In addition to acting as a physical barrier, $Ca(OH)_2$ dressing may both prevent root canal reinfection and interrupt the nutrient supply to remaining bacteria (Siqueira & Lopes 1999). Its high pH (around 12.5) has a destructive effect on cell membranes and protein structure (Spangberg 1994). To be effective against bacteria located inside the dentinal tubules, the hydroxyl ions from $Ca(OH)_2$ should diffuse into dentine at sufficient concentrations and should exceed the dentine buffering ability, reaching pH levels sufficient to destroy bacteria (Siqueira & Lopes 1999). The time needed for $Ca(OH)_2$ to optimally disinfect the root canal system is still unknown and might be related to the presence or absence of root canal exudate, the type of microorganism involved, location of the microorganism in the root canal system, presence or absence of the smear layer and especially, the degree of susceptibility to the medication (Gomes *et al.* in press).

Chlorhexidine gluconate has been widely used in periodontics due to its antibacterial activity (Gjermo 1974, Lindskog *et al.* 1998). Its use in endodontics has been proposed both as irrigant and intracanal medicament (Delany *et al.* 1982,Vahdaty *et al.* 1993, Jeansonne & White 1994, Siqueira & Uzeda 1997, Ferraz *et al.* 2001). Chlorhexidine has inhibitory effects on bacteria commonly found in endodontic infections (Cervone *et al.* 1990), acting against Gram-positive and Gram-negative microorganisms (Waler 1990). One of the mechanisms that explains its efficacy is based on the interaction between the positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall, which allows the chorhexidine molecule to penetrate into the bacteria with toxic effects (Lindskog *et al.* 1998, Hugo & Longworth 1964).

The purpose of this study was to assess *in vitro* the effectiveness of 2% chlorhexidine gluconate gel and $Ca(OH)_2$, separately and combined, as intracanal medicaments, in cylindrical specimens of bovine root dentine against *E. faecalis.*

Materials and methods

The method used was a modification of the one previously described (Haapasalo & Ørstavik 1987). One hundred and eighty intact freshly extracted bovine maxillary central incisors with complete root formation, measuring approximately 30 mm, were selected for study. The teeth were cleaned with periodontal curettes to remove periodontal tissues and bone. The specimens were kept in 0.5% sodium hypochlorite solution for no longer than 7 days.

Specimen preparation

The apical 5.0 mm and coronal two-thirds were removed from each tooth with a double-face cylindrical diamond (ref. 7020, KG Sorensen, São Paulo, SP, Brazil), at low speed, under water cooling. This left an approximately 18-mm-long specimen with a central pulp space. Each specimen then was divided from the apical end into three blocks of 4 mm length, simulating the cervical, medium and apical root thirds. Only the middle segments were used that had an external diameter of approximately 6 mm (Fig. 1).

Root cementum was removed using long cylindrical diamond burs (ref. 3101, KG Sorensen, São Paulo, SP, Brazil), in a high-speed handpiece, under water cooling (Haapasalo & Ørstavik 1987). Standardization of root canal diameters was achieved using round carbide burs with a 2.1-mm diameter (ISO 021, Maillefer Dentsply,

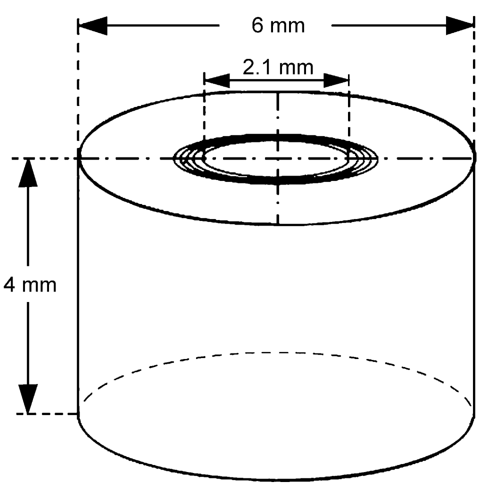

Figure 1 Diameters of the dentine tubes (middle segment).

Ballaigues, Switzerland), in a low-speed handpiece. The teeth and dentine specimens were stored in tap water at all times to avoid dehydration.

Dentine treatment to remove the smear layer

The dentine tubes were individually placed in bijoux bottles containing 3 mL of 5.25% NaOCl, which were transferred to an ultrasonic bath for 10 min. The specimens were then washed with running tap water for 1 h to remove NaOCl residues (Perez *et al.* 1993).

The specimens were placed in bijoux bottles containing 17% EDTA (with pH 7.2) and were again ultrasonicated for 10 min. Next, they were washed for 1 h in running tap water. The efficiency of the method was observed in a pilot study using SEM examination of standard images of the middle third of the root canals at ×2000 (15 kV) magnification, where it was possible to verify the presence of open dentinal tubules.

Specimen sterilization

The dentine tubes were individually placed in bijoux bottles containing 3.0 mL of Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) medium and autoclaved at 121 °C, 1 atm, for 15 min. They were then kept in an incubator at 37 °C for 24 h to check the efficacy of the sterilization treatment.

Contamination with *Enterococcus faecalis*

Isolated 24-h colonies of pure cultures of *E. faecalis* (ATCC 29212) grown on 5% defibrinated sheep blood + BHI agar plates were suspended in 5.0 mL of BHI. The cell suspension was adjusted spectrophotometrically to match the turbidity of 1.5×10^8 CFU mL^{-1} (equivalent to ≠0.5 McFarland standard).

The bijoux bottles containing each specimen were opened under laminar flow. Sterile pipettes were used to remove 2.0 mL of sterile BHI and to replace it with 2.0 mL of the bacterial inoculum. The bottles were closed and kept at 37 °C for 7 days, with the replacement of 1.0 mL of contaminated BHI for 1.0 mL of freshly prepared BHI every 2 days, to avoid medium saturation. The turbidity of the medium during the incubation period indicated bacterial growth. The purity of the cultures was confirmed by Gram staining, catalase production, colony morphology on BHI agar + blood and by the use of a biochemical identification kit (API 20 Strep, bioMérieux SA, Marcy-l'Etoile, France).

Bacterial penetration into the dentinal tubules using this technique was confirmed by SEM in a pilot study (Fig. 2).

Antibacterial assessment

Following the contamination period, each specimen was removed from its bijoux bottle under aseptic conditions, and the canal irrigated with 5.0 mL of 0.85% sterile saline and dried with sterile paper points. The outer surfaces of the specimens were covered with nail varnish, in order to prevent contact of the medicament with the external surface. The specimens were fixed at the bottom of wells of 24-well cell culture plates (Corning, NY, USA, ref. no. 3524; well volume 3.2 mL) with decontaminated sticky wax, which also obliterated the apical surface of the root canal.

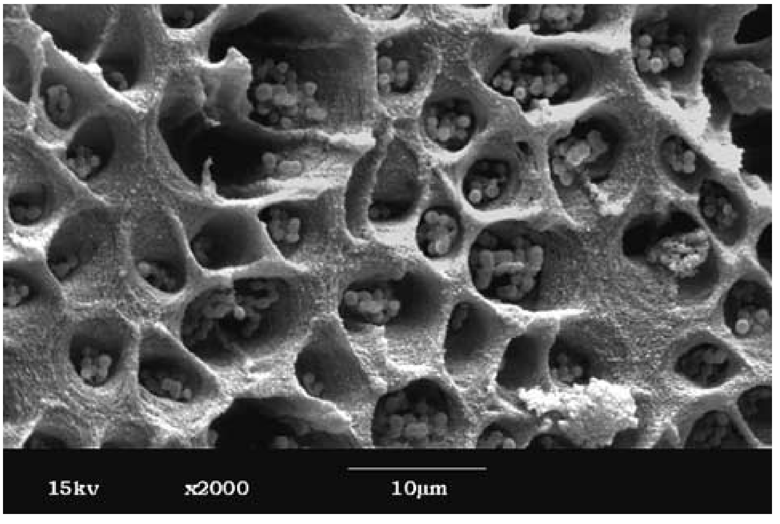

Figure 2 Contamination of the bovine dentinal tubules.

The wells were then filled with agar at 46°C until it reached the upper surface of the dentine specimens. Finally, the intracanal medicaments were applied to the canal lumen with the help of sterile 3.0-mL plastic syringes and 27-gauge needles (Becton Dickinson Indústria Cirúrgica Ltda, Curitiba, PR, Brazil), until the canals were totally full.

The 180 specimens were divided into four groups, according to the intracanal medicament used, as follows:

- Group 1 (45 specimens): 2% chlorhexidine gel.
- Group 2 (45 specimens): $Ca(OH)_2$ paste in a viscous vehicle (polyethyleneglycol 400) (1 : 1).
- Group 3 (45 specimens): 2% chlorhexidine gel + $Ca(OH)_2$ paste (1 : 1). A total of 0.20 g of each medication was weighted and mixed well.
- Group 4 (control group, 45 specimens): BHI suspension. Each specimen had the canal lumen filled with 40 µL BHI medium, which was replaced by the same amount every 2 days in order to maintain bacterial growth.

Following the placement of all intracanal medicaments inside the dentine specimens, they were incubated at 37°C, according to the experimental periods: 1, 2, 7, 15 and 30 days. Each period of time was tested in triplicate and the whole experiment was repeated thrice.

The pH of chlorhexidine and $Ca(OH)_2$ pastes, alone and combined, was measured (Procyon, digital pH meter model AS 720, electrode A 11489, Procy Instrumental Científica, São Paulo, SP, Brazil).

Dentine samples

At the end of the experimental periods, the specimens were removed from the culture wells and the canals irrigated with 5.0 mL of 0.85% sterile saline to remove all medicament and dried with sterile paper points.

Each specimen was mounted in a sterile aluminium apparatus (Fig. 3), and low-speed burs with increasing diameters (ISO 0.23, 0.25, 0.27, 0.29. Maillefer Dentsply), held in a low-speed electrical motor (Maillefer Dentsply), were used for the sequential removal of dentine to a depth of 0 to approximately 0.4 mm (400 µm) (Fig. 4). Each bur removed approximately 0.1 mm (100 µm) of dentine around the canal, weighing approximately 4 mg. In order to standardize the volume of dentine chips removed with each bur, the BHI bottles were weighed before and after dentine collection. The dentine chips produced by each bur, as well as the remaining dentine specimen (cylinder), were immediately collected into individual bijoux bottles containing 3.0 mL of freshly prepared BHI + neutralizers to avoid continued action of the medicaments. The neutralizer for $Ca(OH)_2$ was 0.5% citric acid, whilst 0.5% Tween 80 + 0.07% lecithin was used for chlorhexidine alone or combined with $Ca(OH)_2$ (Siqueira *et al.* 1998). The bottles were then incubated at 37°C for a period of 30 days, and observed for daily microbial growth visualized by medium turbidity.

The purity of the positive cultures was confirmed by Gram staining, catalase production, colony morphology on BHI blood agar and by the use of a biochemical identification kit (API 20 Strep, bioMérieux SA, Marcy-l'Etoile, France).

Statistical analysis

The data were statistically analysed using Fisher's exact test to check the frequency of positive bacterial cultures

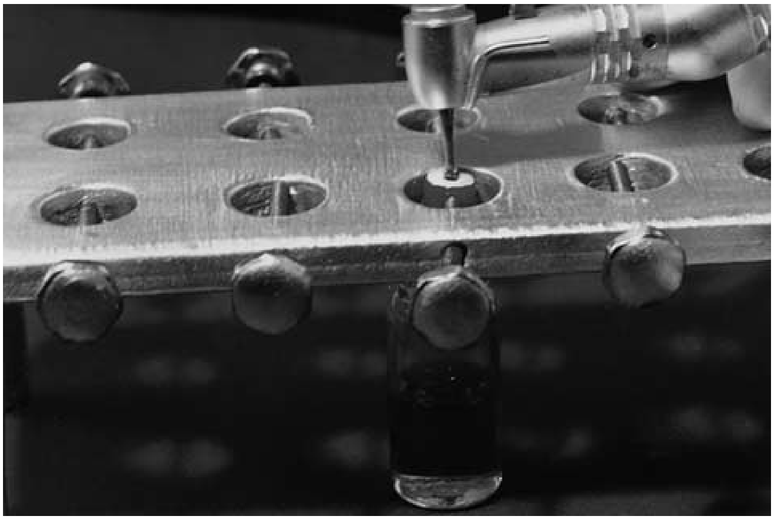

Figure 3 Experimental apparatus.

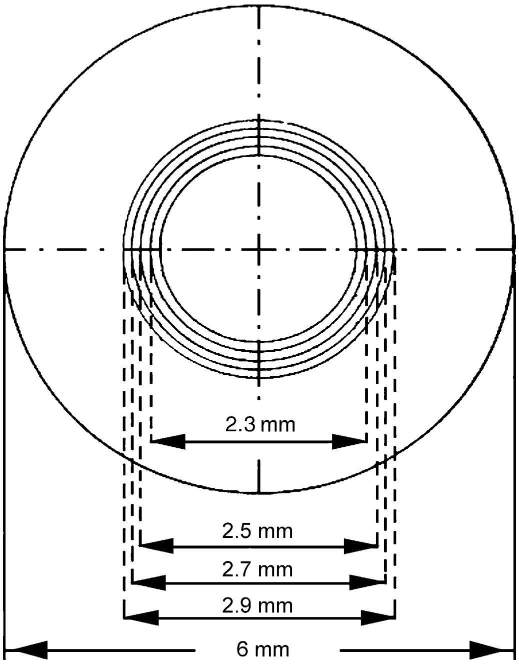

Figure 4 Sequential removal of the dentine chips.

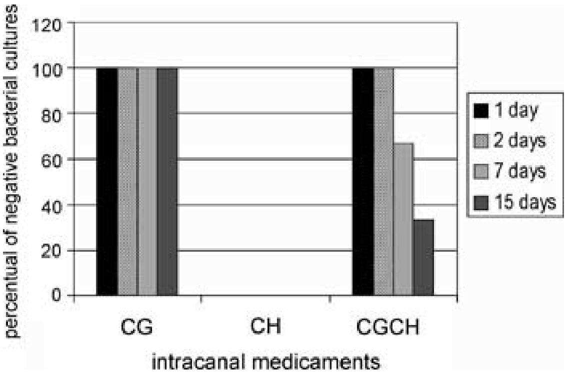

Figure 5 Antibacterial activity of 2% chlorhexidine gel (CG), calcium hydroxide (CH) and the combination of both (CGCH) against *Enterococcus faecalis*.

achieved by the medicaments on the *E. faecalis* contaminated bovine dentine. The significance was established at 5% level ($P = 0.05$).

Results

The pH of chlorhexidine and $Ca(OH)_2$ pastes, alone and combined, were: chlorhexidine gel, pH 7.0; $Ca(OH)_2$ paste, pH 11.0 and clorhexidine gel + $Ca(OH)_2$, pH 12.8.

Antibacterial action of the medicaments against *Enterococcus faecalis* in relation to the experimental times

The bottles considered to have positive bacterial growth were those which presented medium turbidity higher than the turbidity of a $\neq 0.5$ McFarland standard (1.5×10^8 CFU mL^{-1}). Such microbial growth was achieved by burs of sizes 2.3–2.9. Therefore, the results in Table 1 are related to the independent reading of the bur diameters, i.e. 45 readings were completed for each medicament at each experimental time, excluding the remaining dentine specimen.

The control group showed viable bacteria at all experimental times, confirming the efficiency of the methodology. On the other hand, cultures that were not turbid at the 72-h observation period remained negative to the end of the observation period of 30 days.

Chlorhexidine gel alone inhibited the growth of the *E. faecalis* after 1, 2, 7 and 15 days. Calcium hydroxide was ineffective at all experimental times. The combination of chlorhexidine and $Ca(OH)_2$ was effective after 1 and 2 days; however, the antibacterial activity reduced between 7 and 15 days, showing antibacterial action in 66.6 and 33.3% of the specimens, respectively (Fig. 5). After 30 days, all specimens showed bacterial growth with all medicaments.

Comparison of antibacterial activity between the medicament at all time period

At days 1 and 2, chlorhexidine gel alone or in combination with $Ca(OH)_2$ demonstrated 100% antibacterial

Table 1 Percentage of negative bacterial cultures by experimental period

Medicaments	Experimental times				
	1 day*	2 days*	7 days*	15 days*	30 days
2% Chlorhexidine gel	100	100	100	100	0
Calcium hydroxide	0	0	0	0	0
Chlorhexidine + calcium hydroxide	100	100	66.6	33.3	0

*Statistical significance amongst the medicaments (Fisher's exact test).
Results from all depths analysed (excluding the remaining dentine specimens) are combined. Number of tests for each medication in all experimental times = 18.

Medicaments	Experimental times				
	1 day	2 days*	7 days*	15 days*	30 days
2% Chlorhexidine gel	0	100	100	100	0
Calcium hydroxide	0	0	0	0	0
Chlorhexidine + calcium hydroxide	0	0	66.6	33.3	0

*Statistical significance amongst the medications (Fisher's exact test).
Number of tests for each medication in all experimental times = 9.

Table 2 Percentage of negative bacterial cultures by experimental period over the remaining dentine specimens (cylinders)

action. After 7 days and up to 15 days, chlorhexidine gel maintained the antibacterial action and was statistically different when compared to other medicaments ($P < 0.05$). In the same period of time, the combination of chlorhexidine + Ca(OH)2 demonstrated reduced antimicrobial action from 66.6% of specimens up to 7 days, to 33.3% of specimens up to 15 days. This was significantly greater than $Ca(OH)_2$ but smaller than chlorhexidine gel ($P < 0.05$). By day 30, bacterial growth was observed at all dentine depths and in all groups.

Comparison of antibacterial action between the medicaments over the remaining dentine specimens

The results in Table 2 are related to the reading of negative bacterial cultures promoted by the medicaments over the remaining dentine specimens at all experimental times. The specimens supported bacterial growth at day 1 and day 30 of contact with the medicaments. Calcium hydroxide did not have an antibacterial action at any experimental time. After day 2, chlorhexidine gel had 100% of antibacterial action over the remaining dentine specimen (cylinder). This was maintained up to 15 days, and was statistically more efficient than the other medicaments ($P < 0.05$).

The combination of chlorhexidine + $Ca(OH)_2$ showed 100% bacterial growth up to the second day. However, after 7 days an antibacterial action was observed in 66.6% of the specimens, and up to 15 days in 33.3% of the specimens.

Discussion

Several models have been proposed in the literature for the study of dentine infection and most of them use *E. faecalis* as the microorganism of choice (Meryon *et al.* 1986, Ørstavik & Haapasalo 1990, Vahdaty *et al.* 1993, Siqueira & Uzeda 1996, Tanriverdi *et al.* 1997, Komorowski *et al.* 2000, Ferraz *et al.* 2001, Gomes *et al.* 2001a,b). *Enterococcus faecalis* is a facultative Gram-positive anaerobic coccus, which is related to persistent root canal infections (Engström 1964, Haapasalo *et al.* 1983, Gomes *et al.* 1996b, 2001a,b, Molander *et al.* 1998, Peciuliene *et al.* 2000, Pinheiro *et al.* in press). The ATCC 29212 strain has been used in several *in vitro* studies in order to test the antimicrobial action of intracanal medication (Haapasalo & Ørstavik 1987, Ørstavik & Haapasalo 1990, Barbosa *et al.* 1997, Siqueira & Uzeda 1997, Komorowski *et al.* 2000).

Bovine incisor teeth were used in the present study because their dentinal tubules are very similar to human teeth in quantity, size, diameter, morphology and density. Moreover, these teeth are simple to obtain and their size makes handling easier (Haapasalo & Ørstavik 1987, Ida *et al.* 1989, Ørstavik & Haapasalo 1990, Heling *et al.* 1992a,b).

In vitro studies have shown that bacteria are usually killed rapidly in direct contact with various medicaments, even in high dilutions (Spangberg *et al.* 1973). However, data on the efficacy of endodontic medication against specific bacteria *in vivo* are limited (Haapasalo & Ørstavik 1987).

In the present investigation, the dentine chips were collected in BHI broth, and not in saline or in transport medium such as RTF or VMGAIII, as this medium is able to support the growth of *E. faecalis* (Gomes *et al.* 2001a,b). The methodology used was a modification of the one previously described by Haapasalo & Ørstavik (1987); counts of colony forming units (CFU) were not included in this study. However, the McFarland standard was used to determine the turbidity of the microbial growth. Cultures that were not turbid at the 72-h observation period remained negative to the end of the observation period of 30 days.

Even though $Ca(OH)_2$ does have some antibacterial action, under the experimental conditions it was not able to kill and eliminate sufficient cells of *E. faecalis* at any time. This is in accordance with the findings of other reports (Haapasalo & Ørstavik 1987, Ørstavik & Haapasalo 1990, Siqueira & Uzeda 1996, Tanriverdi *et al.* 1997, Estrela *et al.* 1999, Haapasalo *et al.* 2000). Other *in vitro* studies have also indicated that enterococci may remain viable after relatively long exposures to $Ca(OH)_2$ (Stevens

& Grossman 1983, Safavi *et al.* 1990, Tanriverdi *et al.* 1997, Gomes *et al.* 2001a,b).

The contradictions between clinical and *in vitro* findings might suggest that other aspects are involved. One mechanism that can explain its *in vivo* antimicrobial activity is the ability of $Ca(OH)_2$ to absorb carbon dioxide in the root canals (Kontakiotis *et al.* 1995), which is essential for bacteria such as *Capnocytophaga, Eikenella* and *Actinomyces* spp. (Sundqvist 1992) and is provided by bacteria such as *Fusobacterium, Bacteroides, Porphyromonas* and *Streptococcus* spp. If $Ca(OH)_2$ absorbs carbon dioxide, CO_2-dependent bacteria will not survive. Therefore, the use of an intracanal medicament will disturb established nutritional interrelationships, eliminating some bacteria that might be essential to the growth of others, or leaving some bacteria whose presence will prevent the growth of others (Gomes *et al.* in press). Furthermore, the recovery of microorganisms is directly associated with the sampling, transport and identification techniques used (Gomes *et al.* 1994, Reit *et al.* 1999). To date there is no single method able to recover every microorganism found in a site, and the use of molecular identification techniques together with culture is recommended. However, molecular techniques cannot differentiate between viable and dead cultures. On the other hand, using traditional culture techniques, a minimum concentration of microorganism is necessary for their isolation, and hence for their recognition in the clinical situation (Gomes *et al.* 1995).

Chlorhexidine gluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components (Greenstein *et al.* 1986). At low concentrations of chlorhexidine, small molecular weight substances will leak out, resulting in a bacteriostatic effect. At higher concentrations, chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking (Kontakiotis *et al.* 1995).

Chlorhexidine in a gel formulation has other important proprieties, such as low toxicity to the periapical tissues (Greenstein *et al.* 1986), viscosity that keeps the active agent in contact with the root canal walls and dentinal tubules, and water solubility (Ferraz *et al.* 2001, Gomes *et al.* 2001b).

In the present study, 2% chlorhexidine gel showed antimicrobial activity against *E. faecalis* after 1, 2, 7 and 15 days. This result agrees with those of others (Delany *et al.* 1982, Heling *et al.* 1992a, Barbosa *et al.* 1997, Siqueira & Uzeda 1997, Haapasalo *et al.* 2000, Komorowski *et al.* 2000), even though such studies utilized chorhexidine in the liquid or gel formulation, and in other concentrations such as 0.05, 0.12, 0.2 and 0.5%.

The combination of chlorhexidine gel + $Ca(OH)_2$ inhibited 100% the growth of *E. faecalis* after 1 and 2 days of contact. However, its antimicrobial activity began to decrease after day 7, and by day 30 the antibacterial action was not enough to eliminate bacterial cells, allowing the medium became turbid.

The antibacterial action of the combined medicaments during the first 2 days might be due to its high pH (12.8) (chlorhexidine, pH 7.0; $Ca(OH)_2$, pH 11), suggesting an increase of the ionized capacity of the chlorhexidine molecule. It is interesting that the majority of the bacteria present in the root canal system grow best at a pH around 6.5–7.5, and only few microorganisms are able to survive at high pH.

According to Fava & Saunders (1999), the vehicles carrying the medicaments play the most important role in the biological action of $Ca(OH)_2$, which is determined by the velocity of ionic dissociation in Ca^{2+} and OH^- ions. The gel base of the chlorhexidine, natrosol, is a methylcellulose that is classified as an aqueous vehicle (like polyethylenoglycol, Spangberg 1994), also explaining the high pH of the combined chorhexidine + $Ca(OH)_2$.

Between days 7 and 15, antibacterial activity was observed in 66.6 and 33.3% of the specimens, respectively, suggesting that even though the combination increased the pH, $Ca(OH)_2$ may have decreased the antibacterial activity of the chlorhexidine, possibly due to a loss in its capacity to adhere to the bacterial cell wall. This could occur because there is a competition between the positive charge of the chlorhexidine molecule and calcium ions for common binding sites such as the negatively charged phosphate groups on the bacterial cell wall (Rölla & Melsen 1975, Bonesvoll 1977a,b, Waltimo *et al.* 1999). Other hypotheses may be the buffer effect that dentine exerts over $Ca(OH)_2$, reducing its antibacterial action (Haapasalo *et al.* 2000). Consequently, the combination of both medicaments had a decreasing antibacterial activity.

The antibacterial action of 2% chlorhexidine gel over the remaining dentine specimen (cylinder) after 2, 7 and 15 days of contact suggests the penetration of the medicament inside the dentinal tubules to a depth over 0.4 mm. On the other hand, the combined chlorhexidine + $Ca(OH)_2$ showed antibacterial action over the remaining dentine specimens only between 7 and 15 days, in 66.6 and 33.3% of the specimens, respectively.

After 30 days of contact, both chlorhexidine as well as the combination of chlorhexidine + $Ca(OH)_2$ did not

eliminate and kill enough *E. faecalis* cells, allowing the growth of those that had remained viable inside the dentinal tubules, but in a level too low for culture identification. It could be that negative cultures do not always mean absence of microorganism, as the cells can be present in low numbers, making it difficult to detect their presence by use of microbial culture methods. Therefore, in the first 15 days, *E. faecalis* was present at a low level due to the antibacterial action of chlorhexidine alone or in combination. After 30 days, there was a loss of the antibacterial activity of the medicaments, allowing their growth and detection in the culture medium.

The results of the present study suggest that chlorhexidine gel has a greater antibacterial activity against *E. faecalis* than $Ca(OH)_2$, but it loses this property if used for longer periods.

Acknowledgements

This work was supported by the Brazilian agencies FAPESP (2000/13689-7; 1996/5584-3), CNPq (520277/99-6) and CAPES.

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